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Platelet inhibition by low-dose aspirin but not by clopidogrel reduces the axon-reflex current-induced vasodilation in humans

P. Rousseau,^{1*} M. Tartas,^{1*} B. Fromy,^{4*} A. Godon,² M.-A. Custaud,^{1,3} J. L. Saumet,^{1,4} and P. Abraham^{1,3}

¹Laboratory of Vascular Investigations, ²Laboratory of Haemostasis University Hospital of Angers, France, ³Integrative Neurovascular Biology, UMR Centre National de la Recherche Scientifique (CNRS) 6214 - Institut National de la Santé et de la Recherche Médicale 771, Medical School, University of Angers, France; and ⁴Neurovascular Interactions Formation de Recherche en Evolution CNRS 3075, University of Lyon 1, France

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Rousseau P, Tartas M, Fromy B, Godon A, Custaud M-A, Saumet JL, Abraham P. Platelet inhibition by low-dose aspirin but not by clopidogrel reduces the axon-reflex current-induced vasodilation in humans. *Am J Physiol Regul Integr Comp Physiol* 294: R1420–R1426, 2008. First published February 6, 2008; doi:10.1152/ajpregu.00810.2007.—We previously showed a prolonged inhibition of current-induced vasodilation (CIV) after a single oral high dose of aspirin. In this study, we tested the hypothesis of platelet involvement in CIV. Nine healthy volunteers took 75 mg aspirin/day, 98 mg of clopidogrel bisulfate/day, or placebo for 4 days. CIV was induced by two consecutive 1-min anodal current applications (0.08 mA/cm²) through deionized water with a 10-min interval. CIV was measured with laser Doppler flowmetry and expressed as a percentage of baseline cutaneous vascular conductance: %C_b. In a second experiment in 10 volunteers, aspirin and placebo were given as in *experiment 1*, but a 26-h delay from the last aspirin intake elapsed before ACh iontophoresis and postocclusive hyperemia were studied in parallel to CIV. In *experiment 1*, the means \pm SE amplitude of CIV was 822 ± 314 , 313 ± 144 , and $746 \pm 397\%$ C_b with placebo, aspirin ($P < 0.05$ from placebo and clopidogrel), and clopidogrel (NS from placebo), respectively. In *experiment 2*, CIV impairment with aspirin was confirmed: CIV amplitudes were 300 ± 99 , and $916 \pm 528\%$ C_b under aspirin and placebo, respectively ($P < 0.05$), whereas vasodilation to ACh iontophoresis (322 ± 74 and $365 \pm 104\%$ C_b) and peak postocclusive hyperemia (491 ± 137 and $661 \pm 248\%$ C_b) were not different between aspirin and placebo, respectively. Low-dose aspirin, even 26 h after oral administration, impairs CIV, while ACh-mediated vasodilation and postocclusive hyperemia are preserved. If platelets are involved in the neurovascular mechanism triggered by galvanic current application in humans, it is likely to occur through the cyclooxygenase but not the ADP pathway.

microcirculation; axon reflex; platelets; skin

A SIGNIFICANT INCREASE in skin blood flow (SkBF) has been observed in response to non-noxious galvanic current application in humans. This current-induced vasodilation (CIV) has been described as the result of an axon reflex (3) and disappears in locally anesthetized or chronically capsaicin-treated skin. Then CIV depends on capsaicin-sensitive fibers and is an

interesting model of the neurovascular interaction following non-noxious stimulation (11, 16). Prostaglandins are synthesized by cyclooxygenases (COX) and play a key role as mediators in the vascular response observed during CIV. COX are expressed in a large variety of human tissues, including endothelium, smooth muscles, nerves, and platelets (29). We recently reported a long-lasting inhibition of CIV (>5 days) following a single high (1,000 mg) oral dose of aspirin (11, 12, 40), which irreversibly blocks both isoforms of COX (COX-1 and COX-2). COX of neuronal origin does not seem responsible for this long-lasting effect (13). This long-lasting inhibition of CIV is consistent with the time required to resynthesize unblocked platelets after oral single-dose aspirin leading to the hypothesis that platelets participate in the vascular response to CIV. Indeed, a single oral high dose of aspirin irreversibly inactivates the platelet COX pathway for the duration of the life of the platelets (~10 days) (1, 36). Indomethacin, a nonspecific COX inhibitor devoid of effect on vanilloid receptors and acid-sensing ion channels (41), abolished CIV, confirming that the inhibition of CIV by aspirin likely resulted from its effect on COX (and not on vanilloid receptors or on acid-sensing ion channels). Furthermore, the specific COX-2 inhibitor, celecoxib, failed to affect CIV, suggesting that CIV is mainly a COX-1-dependent phenomenon (39).

Together, with the long-lasting effect of a single oral high dose of aspirin, the fact that COX-1 isoform participates in CIV raises the question of a possible platelet involvement in the axon-reflex CIV. Although there is, to date, no in vivo proof of a direct platelet-mediated vasodilation in humans, the hypothesis of a platelet involvement in axon-reflex vasodilation and other vasodilator mechanisms has previously been explored in vitro (15, 23, 31). Those authors provided evidence for a direct in vitro platelet-mediated endothelium-dependent vasodilation in precontracted arteries but mainly by the ADP pathway.

To investigate whether the platelet COX and ADP pathways are involved in vivo in CIV, we inhibited platelet function by aspirin (a platelet COX inhibitor) or clopidogrel (a platelet ADP-receptor inhibitor). Our hypothesis was that if platelets

* These three authors contributed equally to this article.

Address for reprint requests and other correspondence: P. Abraham, Laboratory of Vascular Investigations, Univ. Hospital, 49933 Angers cedex 09 France (e-mail: piabraham@chu-angers.fr).

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are involved, both clopidogrel and low-dose aspirin would impair the current-induced vasodilation.

MATERIALS AND METHODS

Healthy nonsmoking volunteers without clinical signs or risk factors for neural and vascular disease were recruited to participate in this institutionally approved study conducted in accordance with the Declaration of Helsinki and registered in the U.S. National Institutes of Health Web site under reference NCT00152724. Before their participation, all subjects were informed of the methods and procedures and gave their written informed consent to participate. Eligible subjects were enrolled after medical interview and investigations, including a normal electrocardiogram, an upper/lower limb Doppler velocimetry, and a blood sample to confirm that platelet function was normal, according to our laboratory standard. Volunteers were not allowed to take any other drugs than those proposed in the protocol in the 3 wk before enrollment and throughout the whole period of the study.

Treatments

Using nonidentifiable capsules, three treatments were performed, each over 4 days. Treatments consisted of a daily oral dose of either 1) aspirin (75 mg/day: Kardegic, Sanofi-Synthelabo, France) or 2) clopidogrel bisulfate (98 mg/day: Plavix, Sanofi-Synthelabo, France) or 3) placebo (lactose 0.21 mg/day, Chemistry of Angers Hospital, France), taken at 7:00 AM every morning. Previous studies have shown that 98 mg of clopidogrel bisulfate per day induces a significant inhibition of platelet aggregation induced by ADP within 3 to 5 days (8, 37).

The order of the treatments was chosen randomly, and subjects and investigators were double blinded as to the nature of the treatment. Experimental trials in the same subject were separated by a minimum interval of 3 wk. Intake and efficacy of the drugs were assessed using platelet aggregation tests.

Platelet Aggregation Study

A blood sample, taken from an antecubital vein at enrollment and just before CIV assessment, was withdrawn into a tube containing 0.1 M sodium citrate (BD Vacutainer, Plymouth, UK).

Platelet aggregation was measured by light transmission (PACKS 4, Helena, Claremont, Ontario, Canada) in stirred (1,000 rpm) platelet-rich plasma. The platelet-rich plasma was prepared by the centrifugation from whole blood at 1,000 g for 7 min and platelet-poor plasma by the centrifugation of the platelet-rich plasma at 3,400 g for 15 min. The platelet count was adjusted to 250,000 platelets/ μ l with platelet-poor plasma; platelet-rich plasma, and platelet-poor plasma were used to set light transmission to 0% and 100%, respectively.

Aggregation was measured as the slope of the aggregation after the addition of the agonist and expressed as a percentage of total aggregation per second.

Aggregation responses were determined in 0.3-ml sample of platelet-rich plasma. Aggregation was stimulated with 2 and 1 μ mol/l ADP (Helena, Claremont, Ontario, Canada) to test the ADP pathway and 0.3 mg/ml arachidonic acid (Helena, Claremont, Ontario, Canada) to test the COX pathway.

With aspirin, the inhibition of arachidonic acid-induced platelet aggregation is almost 100%, while with clopidogrel, the inhibition of ADP-induced aggregation never reaches 100%, is dose and time dependent, and at steady state is in the range of 40 to 60% (33). Then, the efficacy of treatments was defined as a decrease of at least 50% in platelet aggregation induced by arachidonic acid and as a decrease of at least 20% in platelet aggregation induced by ADP (as compared with their reference values) for aspirin and clopidogrel, respectively, compared with individual baseline values. Patients not fulfilling these criteria were not enrolled in the study.

The aggregation study was not used to argue for the participation of platelets in microvascular responses but was aimed at confirming that any absence of drug effects on CIV was not related to an ineffective dose or to nonadherence of the volunteers in following the treatment regimen.

Protocol 1

Nine healthy volunteers (7 males, 2 females), 27.6 ± 2.8 (mean \pm SD) years, height: 177.3 ± 7.2 cm, weight: 68.9 ± 7.4 kg were enrolled in the first protocol.

Treatments were started 4 days before each experiment. Subjects received aspirin, clopidogrel, and placebo treatments in a double-blinded random order, with an interval of at least 3 wk between drug treatments. The last capsule was taken at 7:00 AM on the day of microvascular investigation. Blood sample collections were performed at 9:00 AM to assess platelet aggregation and drug efficiency. The microvascular studies for CIV were started between 9:10 AM and 9:30 AM. Thus, the last drug capsule intake took place ~ 2.25 h before the start of the microvascular tests.

To avoid any local inflammatory effects of the venous puncture on CIV, assessment was performed on the arm contralateral to blood sampling.

Protocol 2

We aimed to test whether any impairment of CIV seen in *protocol 1* resulted from an impairment of endothelium-dependent vasodilation by aspirin, although at such very low doses that endothelial function was expected to be unimpaired. Previous studies have shown that, after aspirin intake, endothelial cells fully recover their cyclooxygenase activity within 24 h (18, 19, 22). Thus if any impairment of endothelial or smooth muscle COX had occurred despite the very low dose of aspirin used in *protocol 1*, a 24-h delay in the assessment of CIV would allow for the restoration of endothelial and smooth muscle cell cyclooxygenase activity, whereas platelet aggregation would continue to be inhibited. Therefore, in *protocol 2*, we tested ACh-mediated vasodilation and postocclusive hyperemia, and treatment intake was staggered earlier than in *protocol 1*, beginning 5 days and stopping 26 h before the experiment.

Ten healthy young men, 23.0 ± 2.8 years, 179.2 ± 5.1 cm, 73.4 ± 9.2 kg, participated in this second protocol. Volunteers enrolled in the first protocol were not allowed to participate in *protocol 2*.

A first CIV test was performed before treatment. This first test was referred as the test "at enrollment" throughout the text, to avoid any confusion with the terminology for baseline values obtained in the first 2 min of each protocol. Subjects were then given either aspirin or placebo in a double-blind manner.

Blood sample collections were performed at 9:00 AM to assess platelet aggregation and drug efficacy. Microvascular investigations were started within half an hour of the blood sampling and performed on the contralateral arm to avoid any variation arising from potential inflammatory effects on the arm used for sampling.

Assessment of CIV (Protocols 1 and 2)

CIV assessments were performed in a quiet air-conditioned room with the ambient temperature set at $24 \pm 1^\circ\text{C}$. The subjects were placed supine and rested for 15 min before data collection. We studied the effect on SkBF of anodal current application, through deionized water, on the volar aspect of the forearm. This technique has been extensively described elsewhere (10). Two laser-Doppler multifiber probes were used and connected to a laser-Doppler flowmeter (Periflux PF4001, Perimed, Sweden). One probe ("active" probe: PF481.1, Perimed, Sweden) was specially designed to allow for SkBF measurements. The "active" probe has a circular chamber, allowing for the positioning of an adhesive patch designed with a sponge of ~ 1.2 cm² area (PF383, Perimed, Järfälla, Sweden). Before each experi-

ment, the sponge was wet with 0.2 ml of deionized water and the patch, combined with the "active" probe, was fixed to the skin. The patch allowed for current application through the anodal terminal of a 9-V current intensity-regulated supplier (Perioint, Micropharmacology System, PF382 Perimed). The cathodal terminal was connected to an Ag/AgCl disposable electrode (Care 610, Kendall, Neustadt, Germany) fixed 5 cm from the "active" probe. The second laser Doppler probe ("reference" probe: PF408, Perimed), positioned on the same volar aspect of the skin forearm, was used to assess the stability of SkBF at an adjacent unstimulated site. The current application consisted of the transcutaneous delivery of two consecutive 1-min periods of 0.1-mA anodal current separated by a 10-min interval. The first current application is known to induce little vasodilator response, whereas the second current application induces an ample aspirin-sensitive vasodilation (11). Following the second period of current application, data were recorded for 10 min.

Assessment of Endothelial Function (Protocol 2 Only)

Simultaneously to CIV testing, an additional identical laser-Doppler multifiber probe was used to assess ACh-mediated vasodilation (PF481.1, Perimed). The technique has been described elsewhere (26, 44). The sponge of the electrode (PF383, Perimed, Sweden) was wet with 0.2 ml of ACh solution (2% in deionized water), followed by an anodal stimulation (9V, 0.08 mA/cm², 20 s, Perioint, Micropharmacology System, PF382 Perimed). The cathodal electrode (Care 610, Kendall, Neustadt, Germany) was placed 5 cm from the anodal electrode.

For the second protocol, a tourniquet was placed proximal to the position of the electrodes. It was used to cause 3 min of ischemia of the forearm by applying supra-systolic pressure for 3 min. Ischemia was started 10 min after the end of the second period of anodal current application, and postocclusive hyperemia was recorded for an additional 15 min. This was used to assess the maximal values during reperfusion, which has been shown to reflect endothelial function within an individual across a treatment period in a reproducible manner (45).

Pressure Measurement

Throughout the experiments, systemic arterial blood pressure was recorded using a Finapres 2350 (Ohmeda, Englewood CO) positioned on the 2nd or 3rd finger of the hand contralateral to the sites of SkBF measurements.

Recordings and Data Analysis

The signals from the laser-Doppler flowmeters and Finapres were recorded on a computer via an analog to digital converter (Biopac System, Goleta, CA) with a sample frequency of 20 Hz. Data collection started with a 2-min baseline period before the onset of current application.

Because of instantaneous variability resulting from vasomotion, all individual laser-Doppler flowmeter signals were averaged over 15-s intervals throughout each experiment (for CIV, ACh-mediated vasodilation, and postocclusive hyperemia). To take into account possible changes in systemic hemodynamic conditions, SkBF was indexed as cutaneous vascular conductance calculated as the ratio of SkBF, expressed in arbitrary units (AU), to mean arterial blood pressure (mmHg) over the same 15-s interval. Vascular conductance was then expressed in AU/mmHg. Baseline values were calculated as the average over the 1-min baseline period prior to the onset of the first current application. All results were expressed as a percentage of baseline conductance (%C_b).

For CIV, we analyzed the maximal vascular conductance recorded following the second period of current application. For ACh iontophoresis, we studied both the peak conductance (immediately after stimulation) and the plateau conductance (20 min later), since we

previously reported that the plateau, but not the peak, was prostaglandin dependent (14). For postocclusive hyperemia, we recorded the peak value for vascular conductance following deflation of the occlusion cuff.

Statistical Analyses

To detect a decrease of CIV from 600%C_b to 200%C_b with 150 SD, power calculations indicated that the minimal number of subjects to be enrolled for $\alpha = 0.05$ and a 80% power was 6.

Data were expressed as means \pm SE. ANOVA with Tukey post hoc was used to compare treatments one to another in all experiments. Statistical analyses were performed with SPSS V13.0 (LEAD Tech., Charlotte, NC). Further, the Pearson test was used to compare the value at enrollment and under placebo in protocol 2 to estimate the test retest reproducibility of the experiments. A two-tailed *P* value less than 0.05 was considered significant in all statistical analyses.

RESULTS

Protocol 1

Assessment of platelet aggregation. As shown in Table 1, aspirin treatment induced a complete abolition of the arachidonic acid-induced platelet aggregation compared with placebo but had no significant effect on the 2 and 1 μ M ADP-induced platelet aggregation. Clopidogrel treatment induced a significant decrease of the 2 and 1 μ M ADP-induced platelet aggregation (on the average -48 to -70% from value at enrollment). A significant decrease of $\sim 50\%$ in the aggregation to arachidonic acid was also noted. No significant difference was found between the values for platelet aggregation observed under placebo and the values at enrollment.

Assessment of CIV. Compared with baseline values, no significant changes were observed for control SkBF at the reference probe or in mean arterial blood pressure during these experiments. No differences were found in vascular conductance values at rest: 0.12 ± 0.03 , 0.23 ± 0.14 , and 0.17 ± 0.09 AU/mmHg among the placebo, aspirin, and clopidogrel trials, respectively. The two 1-min anodal current applications resulted in significant increases of SkBF corresponding to a CIV of 822 ± 314 , 313 ± 144 , and $746 \pm 397\%$ C_b with placebo, aspirin, and clopidogrel, respectively ($P < 0.01$ vs. rest) as shown in Fig. 1. The CIV observed following aspirin treatment was less than the one observed following both placebo and clopidogrel ($P < 0.05$). The CIV after clopidogrel was not significantly different from placebo ($P = 0.923$).

Protocol 2

Assessment of platelet aggregation. As shown in Table 2, aspirin treatment induced a complete abolition of the arachidonic acid-induced platelet aggregation in six subjects. In the

Table 1. Aggregation velocity at enrollment and under treatment in experiment 1

	At Enrollment	Clopidogrel	Aspirin	Placebo
2 μ M ADP	83 \pm 18	51 \pm 17*†	87 \pm 11	84 \pm 16
1 μ M ADP	53 \pm 17	21 \pm 14*†	60 \pm 11	51 \pm 12
Arachidonic acid	77 \pm 27	41 \pm 17*	0 \pm 0*‡	63 \pm 17

Values are expressed as means \pm SE. * $P < 0.05$ from values at enrollment. † $P < 0.05$ from values under aspirin and placebo. ‡ $P < 0.05$ from values under clopidogrel and placebo.

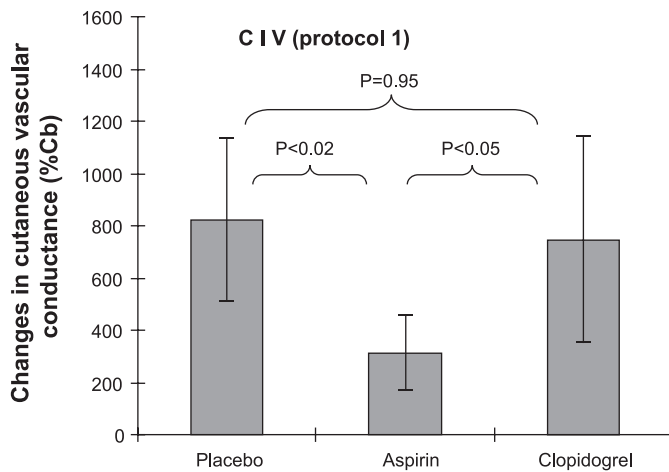


Fig. 1. Values are expressed as means \pm SE for responses of maximal cutaneous vascular conductance to two consecutive applications of 1-min monopolar anodal current (CIV) following placebo, aspirin, or clopidogrel in *protocol 1*. Results are presented as a percentage from baseline value.

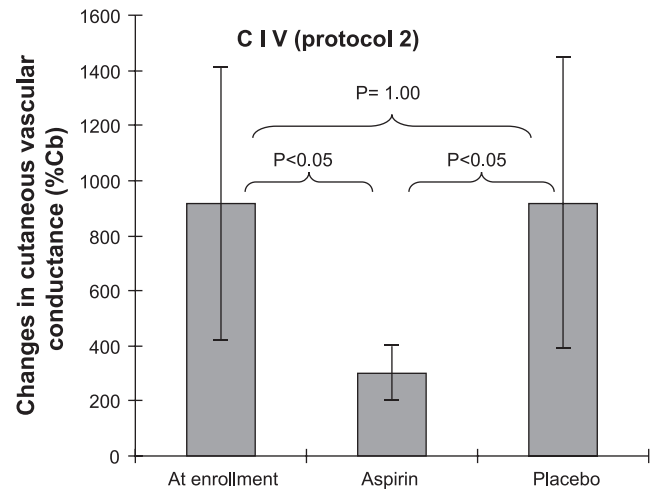


Fig. 2. Values are expressed as means \pm SE for responses of maximal cutaneous vascular conductance to two consecutive applications of 1-min monopolar anodal current (CIV) at enrollment and following aspirin or placebo in *protocol 2*. Results are presented as a percentage from baseline value.

other four subjects, the arachidonic acid-induced platelet aggregation was severely impaired ($\sim 10\%$ of the value at enrollment), which seems consistent with the $\sim 10\%$ resynthesis of platelets after 1 day without aspirin intake. When compared with placebo and the values at enrollment, the effect of aspirin on arachidonic acid-induced platelet aggregation was significant ($P < 0.05$). Placebo showed no difference from the values at enrollment.

Assessment of CIV. Compared with baseline values, no significant changes were observed either for control SkBF at the reference probe or for mean arterial blood pressure during these experiments. Figure 2 represents the mean of the vascular conductances during the second protocol for the anodal current application with deionized water. No differences were found in vascular conductance values at rest: 0.10 ± 0.05 , 0.10 ± 0.05 , and 0.11 ± 0.03 AU/mmHg for the values at enrollment, with aspirin and with placebo, respectively. As in *protocol 1*, the two 1-min anodal current applications were followed by an abrupt vasodilation for both the experiment at enrollment and with placebo (Fig. 2), whereas the vasodilator response was significantly impaired with aspirin pretreatment. Peak responses after the two periods of current application were 915 ± 493 , 300 ± 99 , and $916 \pm 528\%C_b$ for the values at enrollment, with aspirin and with placebo, respectively. No significant difference was seen between placebo and values at enrollment and a significant correlation was found between individual values at enrollment and under placebo: $r = 0.64$ $P < 0.05$. A significant difference was observed between aspirin and

either placebo or values at enrollment ($P < 0.05$), thus confirming the results observed in *protocol 1*.

Assessment of endothelium-dependent vasodilation. No differences were found in vascular conductances at rest: 0.14 ± 0.08 , 0.12 ± 0.06 , and 0.09 ± 0.04 AU/mmHg for the values at enrollment, with aspirin and with placebo, respectively. The peak values following ACh iontophoresis were similar at enrollment, with aspirin and with placebo: 374 ± 145 , 322 ± 74 , and $365 \pm 104\%C_b$, respectively (no significant differences were detected among treatments, $P > 0.05$). Figure 3 represents the mean \pm SE levels of peak vasodilation during ACh iontophoresis. No difference was observed for the plateau values following ACh iontophoresis: 248 ± 117 , 167 ± 40 , and $176 \pm 57\%C_b$ for the values at enrollment, with aspirin and with placebo, respectively. A significant correlation was found between individual values at enrollment and with placebo $r = 0.89$ $P < 0.001$.

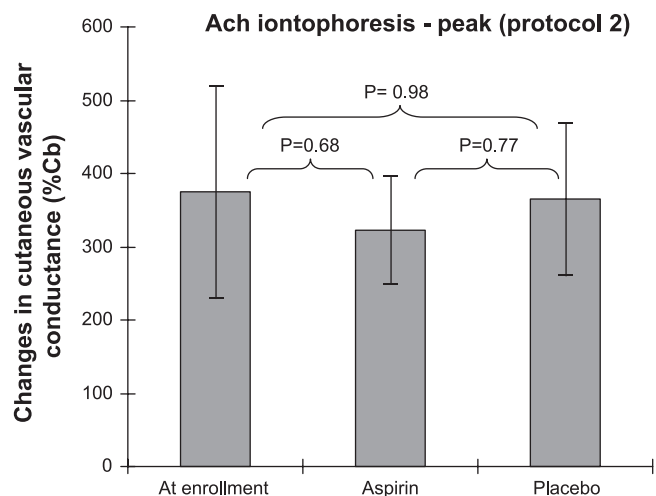


Fig. 3. Values are expressed as means \pm SE for responses of maximal cutaneous vascular conductance during ACh iontophoresis at enrollment and following aspirin or placebo in *protocol 2*. Results are presented as a percentage from baseline value.

Table 2. Aggregation velocity at enrollment and under treatment in experiment 2

	At Enrollment	Aspirin	Placebo
2 μ M ADP	169 \pm 22	183 \pm 23	178 \pm 13
1 μ M ADP	99 \pm 25	120 \pm 21	115 \pm 28
Arachidonic acid	166 \pm 28	6 \pm 8*	176 \pm 11

Values are expressed as means \pm SE. * $P < 0.05$ from values at enrollment and placebo.

Finally, as shown in Fig. 4, we found no difference in the post-occlusive reactive hyperemia between the three groups. Peak values were 545 ± 242 , 491 ± 137 , and $661 \pm 248\%C_b$, respectively, for the values at enrollment, under aspirin and under placebo. A significant correlation was found between individual values at enrollment and under placebo: $r = 0.85$ $P < 0.002$.

DISCUSSION

It is generally accepted that platelets participate in vasoconstriction when activated. Nonetheless, physiological models suggest that platelets might also be involved in the vasodilator mechanisms observed during neurogenic inflammation. However, to date, no *in vivo* evidence existed for a platelet-induced vasodilation, specifically in humans. There are two major findings relevant to this issue from the present study. In *protocol 1*, low-dose aspirin treatment significantly decreased CIV, whereas clopidogrel did not. Thus, if platelets are involved in CIV (a model of neurogenic inflammation), it is likely through the COX and not through the ADP pathway. The second protocol confirmed that impairment of CIV occurred, while ACh-mediated vasodilation and postocclusive hyperemia were preserved.

Following *protocol 1*, the fact that CIV was impaired under aspirin in this study is consistent with previous studies in which we used a single oral high dose of aspirin (10–12). Because this high dose of aspirin induces a systemic inhibition of COX, the identification of the origin of the COX involved in CIV was not possible. In contrast to the high dose, aspirin given at very low doses spares or minimally influences the vascular synthesis of prostaglandins (17, 43). Indeed, at low doses of aspirin (up to 325 mg daily), the systemic vascular endothelium undergoes minimal exposure to aspirin due to its extensive presystemic hepatic metabolism, whereas platelets undergo much greater exposure (30). In parallel to COX inhibition, aspirin increases lipoxygenase-derived metabolites; that is, leukotrienes (27). Most leukotrienes exhibit endothelial-dependent relaxation properties (38). Thus, it is unlikely that the decreased vasodilation to CIV under aspirin is due to the effect of the drug on

leukotrienes bioavailability. Besides its inhibition of the COX pathway (17, 34, 43), it has been suggested that aspirin may interfere with the ADP-induced platelet aggregation (7, 24). However, we observed no apparent change in the platelet aggregation response to ADP with aspirin treatment.

Clopidogrel is a new drug in the recently developed class of thienopyridine derivatives used to block ADP receptors on platelets, blockade that results in an inhibition of platelet aggregation. The active metabolite of clopidogrel has been described as having a pharmacodynamic pattern quite similar to that of aspirin, causing the cumulative inhibition of platelet aggregation on repeated daily administration (32). Both the cumulative nature of the inhibitory effects and the slow rate of recovery of platelet function under clopidogrel cause a permanent defect in a platelet protein that is not reversed during the 24-h dosing interval and can only be replaced as a function of platelet turnover (32). Clopidogrel did not significantly impair anodal CIV, whereas low-dose aspirin did. This occurred although the efficacy of our treatment was in accordance with the maximum inhibitory effects of clopidogrel on ADP-induced aggregation observed at steady-state optimal clopidogrel treatment (33). Platelet aggregation to arachidonic acid was significantly decreased under clopidogrel. This latter finding is consistent with previous reports (4, 35) but, to date, remains unexplained. One possible explanation relates to the observation that the ADP pathway modulates platelet activation mediated by other physiological agonists (9, 28).

In *protocol 2*, the 26 h between the last aspirin intake and microvascular experiments were assumed to allow for a complete recovery of endothelial COX synthesis, if any impairment of this synthesis had occurred in *protocol 1*. As previously discussed, endothelial prostaglandin synthesis was expected to be unimpaired at very low aspirin doses (in the range of 40–80 mg) but not at higher doses (17, 43). Previously, dosages of 486 to 500 mg aspirin were used to block endothelial cyclooxygenase *in vivo* in conduit vessels (2, 21). In the microcirculation 1,000 mg aspirin consistently impairs the late phase of ACh-mediated vasodilation (14). As a result of the very low dose used and of the delay from the last aspirin intake in *protocol 2*, no apparent impairment of endothelium-dependent vasodilation was observed, whereas CIV was impaired. Thus the impairment of CIV did not result from endothelial COX blockade.

The absence of effect of clopidogrel and the inhibitory effect of 75 mg of aspirin on CIV (even 26 h after the last dose), suggest that platelets could be involved in CIV but via the COX rather than the ADP pathway. The absence of CIV under aspirin in *protocol 2*, while aggregation was partly restored, also suggests that partial residual platelet function “normalizing” CIV was not the cause for the absence of CIV impairment with clopidogrel in *protocol 1*.

Study limitation. The possible platelet involvement suggested by the present results is consistent with the absence of effect of the specific COX-2 inhibitor (celecoxib) on CIV, as previously observed (39), since it is well known that most platelet prostaglandins are synthesized by COX-1. In principle, antiplatelet agents, such as aspirin or clopidogrel, do not fully inhibit activation of platelets stimulated by agents other than arachidonic acid or ADP (42). It cannot be excluded that the persistent moderate response under aspirin and the almost

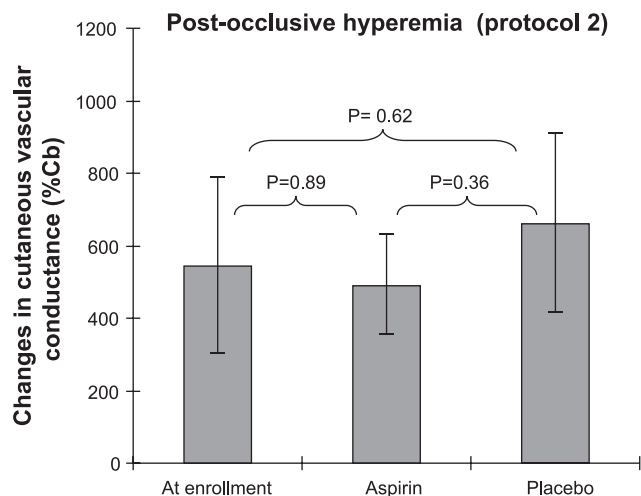


Fig. 4. Values are expressed as means \pm SE for responses of maximal cutaneous vascular conductance during postocclusive hyperemia to 3-min ischemia, at enrollment and following aspirin or placebo in *protocol 2*. Results are presented as a percentage from baseline value.

normal response under clopidogrel resulted from the activation of platelets through another pathway.

Could the fact that clopidogrel did not significantly impair CIV be a type 2 error? This is probably not the case since the *P* value is far from significance, and it is thus unlikely that increasing the number of observations could have allowed reaching the significant level consistently with the number of subjects' calculation.

Last, it is notable that patients included in the first protocol were different from those participating in *protocol 2*. This does not allow for the direct comparison of the results from *protocol 1* to the results of *protocol 2*. Nevertheless, this confirmed that the impairment of CIV observed with aspirin was not specific of the population studied in *protocol 1*.

Perspectives and Significance

These findings indicate that platelets are involved in the neurovascular vasodilation triggered by galvanic current application, mainly through the COX pathway. Aspirin and clopidogrel inhibit platelet aggregation by different pharmacodynamic mechanisms. The glycoprotein IIb/IIIa (GP IIb/IIIa) receptor serves as the final common pathway for platelet aggregation (25). Binding of GP IIb/IIIa receptor results in a highly selective and efficient inhibition of platelet aggregation, independent of the particular platelet-activating mechanism. The effect of GP IIb/IIIa inhibitors on CIV might allow for a more specific demonstration of platelet involvement in this model. However, the risk of severe thrombocytopenia from GP IIb/IIIa inhibitors precludes its acceptance for use in healthy subjects. Furthermore, inhibition of the platelet GP IIb/IIIa receptor enhances the release of platelet-derived nitric oxide (6), a potent vasodilator that could interfere with the expected decreased response to galvanic current application.

In the CAPRIE study, clopidogrel was found to have a significant benefit over low-dose aspirin, specifically in PAD patients, in terms of cardiovascular complications (5). More recently, an inhibitory effect of aspirin but not clopidogrel on arteriogenesis has been shown (20). A relationship of these results to the preservation under clopidogrel but not under aspirin of neurovascular interactions involving platelets is a fascinating but unproven hypothesis for future studies.

Last, our results are of potential clinical significance. Low-dose aspirin is largely used in primary prevention among patients with high risk of cardiovascular disease. There is a high correlation between neuropathy and foot ulcer in diabetic patients. The inhibitory effect of aspirin, even at a very low dose, on the neurovascular response to primary afferent fiber activation could be a major disadvantage of this drug in such patients.

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